

APPENDIX A  
PENDING CLAIMS

4. (Reiterated) The method according to Claim 23, wherein said *Bacillus* strain is *Bacillus* novo species PB92 or a derivative thereof.

5. (Reiterated) The method according to Claim 23, wherein said *Bacillus* strain is an asporogenic alkalophilic *Bacillus* strain.

6. (Reiterated) The method according to Claim 23, wherein the gene encoding said indigenous protease has been deleted by homologous or illegitimate recombination.

7. (Reiterated) The method according to Claim 23, wherein a plasmid comprises said expression cassette.

9. (Amended) The method according to Claim 7, wherein said mutant high alkaline protease [exhibiting altered protease activity] is obtained from *Bacillus* novo species PB92.

10. (Reiterated) The method according to Claim 23, wherein at least one copy of said expression cassette is integrated into the genome of said host.

11. (Reiterated) The method according to Claim 10, wherein said host further contains at least one copy of a plasmid comprising said expression cassette.

12. (Amended) A method of obtaining an alkalophilic *Bacillus* strain having no detectable extracellular high alkaline protease, said method comprising:

transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5' and the 3' flanking regions but not the coding region of gene coding for the high alkaline protease and encoding a replication function, wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with an indigenous gene coding for the high alkaline protease whereby transformants are obtained;

growing said transformants under growth conditions to which the replication function of said cloning vector is sensitive whereby the replication function encoded by said vector is inactivated; and

isolating said transformants identified as having said inactivated replication function and no detectable extracellular high alkaline protease.

13. (Reiterated) The method according to Claim 12, wherein said alkalophilic *Bacillus* strain is *Bacillus* novo species PB92 or a derivative thereof.

## BEST AVAILABLE COPY

14. (Reiterated) An alkalophilic *Bacillus* strain producing a mutant high alkaline protease which is substantially free of expression product of an indigenous extracellular alkaline protease gene, wherein said strain has been obtained by transforming an alkalophilic *Bacillus* strain having no detectable indigenous extracellular high alkaline protease obtained by the method according to Claim 12, 13 or 27 with a plasmid expression vector comprising the mutant high alkaline protease gene.

15. (Reiterated) The *Bacillus* strain according to Claim 14, wherein said alkalophilic *Bacillus* strain is a mutant of *Bacillus* novo species PB92 or a derivative thereof.

16. (Cancelled) The *Bacillus* strain according to Claim 15, wherein said indigenous gene has been deleted by homologous or illegitimate recombination.

19. (Amended) A detergent composition comprising as an active ingredient one or more mutant forms of high alkaline protease [exhibiting altered protease activity], wherein at least one of a said mutant form of high alkaline protease has been prepared according to the method of Claim 23.

23. (Amended) A method for production of a mutated high alkaline protease [exhibiting altered protease activity and] substantially free of indigenous extracellular high alkaline protease, said method comprising:

growing an alkalophilic *Bacillus* strain host substantially incapable of reversion and having no detectable indigenous extracellular protease as a result of deletion of the gene for indigenous extracellular protease transformed with an expression cassette providing for expression of a said mutant high alkaline protease in said host, whereby said mutant high alkaline protease is produced; and

isolating said mutant high alkaline protease.

24. (Amended) A method for preparing a detergent composition, which comprises the step of combining a detergent composition with, as an active ingredient, one or more mutant forms of a high alkaline protease [exhibiting altered protease activity], wherein at least one of a said mutant form of high alkaline protease has been prepared according to the method of Claim 23.

25. (Amended) A method for processing laundry, which comprises the step of contacting said laundry with a detergent composition comprising as an active ingredient one or more mutant forms of a high alkaline protease [exhibiting altered protease activity], wherein at least one of a said mutant form of high alkaline protease has been prepared according to the method of Claim 23.

26. (Amended) A method for production of a mutated high alkaline protease [exhibiting altered protease activity and] substantially free of indigenous extracellular protease, said method comprising:

growing an asporogenous *Bacillus* strain host having a reduced indigenous extracellular protease level as a result of deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of a mutated high alkaline protease [exhibiting altered protease activity] in said host, whereby said mutated high alkaline protease is produced; and

isolating said mutant high alkaline protease.

27. (Amended) A method of obtaining an alkalophilic *Bacillus* strain having no detectable extracellular high alkaline protease, said method comprising:

transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5' and the 3' flanking regions but not the coding region of gene coding for the high alkaline protease and wherein a sufficient amount of said flanking regions is present to provide for illegitimate recombination with an indigenous gene coding for the high alkaline protease whereby transformants are obtained;

growing said transformants under growth conditions to which the replication function of said cloning vector is sensitive whereby the replication function encoded by said vector is inactivated; and

isolating said transformants identified as having said inactivated replication function and no detectable extracellular high alkaline protease.

28. (Reiterated) A method for producing an alkalophilic asporogenic *Bacillus* novo species PB92 of minimal indigenous extracellular protease level, said method comprising:

transforming an alkalophilic asporogenic *Bacillus* [strain] novo species PB92 with a specifically-mutated *Bacillus* novo PB92 alkaline protease.

29. (Reiterated) An alkalophilic *Bacillus* strain producing a mutant high alkaline protease which is substantially incapable of reversion and which is substantially free of expression product of an indigenous extracellular alkaline protease gene.

SVFS01....\03\48403\4230\0238\URSP4275L.010

---

**BEST AVAILABLE COPY**